Evaluation of two Fasciola hepatica faecal egg counting protocols in sheep and cattle

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Supplementary Material – Veterinary Parasitology Journal

Evaluation of two Fasciola hepatica faecal egg counting protocols in sheep and cattle

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Supplementary Table S1: Total number of eggs added to each artificially spiked sheep and cattle faecal sample.

	Concentration of eggs (epg)			
Method	2	5	10	20
Flukefinder®	4 [d]	10 [d]	20 [b]	40 [b]
Becker	NA	25 [d]	50 [b]	100 [b]

[d] = directly spiked into the faecal sample

[b] = made into a batch of 20 before spiking the faeces

Supplementary Material S1

R code for glmm:

```
library(tidyverse)
library(readxl)

# For GLMM:
library("lme4")

spikedFEC$Species <-factor(spikedFEC$Species, levels = c('cattle', 'sheep'))
spikedFEC$Method <-factor(spikedFEC$Method, levels = c('Flukefinder', 'Becker'))
# Add EPG 2:
spikedFEC$EPG <-factor(spikedFEC$EPG, levels = c('10', '5', '20', '2'))
summary(spikedFEC)


# Without EPG2:
spikedmodel <-glm(cbind(eggsrecovered, eggsinsample - eggsrecovered) ~ Species +
Method + EPG, data = spikedFEC %>% filter(EPG!='2'), family = binomial())

# Model including EPG2:
spikedmodel1 <-glm(cbind(eggsrecovered, eggsinsample - eggsrecovered) ~ Species
+ Method + EPG, data = spikedFEC, family = binomial())

## Models with mixed effects to account for differences between samples:
spikedmodel_glmm <- glmer(cbind(eggsrecovered, eggsinsample - eggsrecovered) ~
Species + Method + EPG + (1 | Row), data = spikedFEC %>% filter(EPG!='2') %>%
mutate(Row = 1:n()), family = binomial())

# Model including EPG2:
spikedmodel1_glmm <- glmer(cbind(eggsrecovered, eggsinsample - eggsrecovered) ~
Species + Method + EPG + (1 | Row), data = spikedFEC %>% mutate(Row = 1:n()),
family = binomial())

summary(spikedmodel)
summary(spikedmodel_glmm)
# The random effect is non-zero so we should prefer the GLMM
spikedmodel <- spikedmodel_glmm
```

```
# with 2 EPG:
summary(spikedmodel1)
summary(spikedmodel1_glmm)
spikedmodel1 <- spikedmodel1_glmm

# Confidence intervals (CI)
confint(spikedmodel1)

#CI for model without 2epg
confint(spikedmodel)

# LRT is likelihood ratio tests for interactions (with 2 epg)
anova(spikedmodel1, update(spikedmodel1, .~. + Method*EPG), test='LRT')
anova(spikedmodel1, update(spikedmodel1, .~. + Method*Species), test='LRT')
anova(spikedmodel1, update(spikedmodel1, .~. + EPG* Species), test='LRT')
summary(update(spikedmodel1, .~. + Method*EPG)) # CR added this summary
summary(update(spikedmodel1, .~. + Method*Species)) # CR added this summary
summary(update(spikedmodel1, .~. + EPG* Species))

#Method*EPG
anova(spikedmodel, update(spikedmodel, .~. + Method*EPG), test='LRT')
summary(update(spikedmodel, .~. + Method*EPG))

#EPG*Species
anova(spikedmodel, update(spikedmodel, .~. + EPG* Species), test='LRT')
summary(update(spikedmodel, .~. + EPG* Species))

#Method*Species
anova(spikedmodel, update(spikedmodel, .~. + Method*Species), test='LRT')
summary(update(spikedmodel, .~. + Method*Species))

#anova.glm

# Extract OR and CI:
or <- cbind(OR=exp(fixef(spikedmodel1)), exp(confint(spikedmodel1))[-1,]) %>%
      as_tibble() %>%
      mutate_all(function(x) format(round(x, digits=2))) %>%
      mutate(variable = names(fixef(spikedmodel1))) %>%
      select(variable, everything())
write_excel_csv(or, "or2epg.csv")

# without 2 epg:
or <- cbind(OR=exp(fixef(spikedmodel)), exp(confint(spikedmodel))[-1,]) %>%
  as_tibble() %>%
  mutate_all(function(x) format(round(x, digits=2))) %>%
  mutate(variable = names(fixef(spikedmodel))) %>%
  select(variable, everything())
write_excel_csv(or, "or.csv")
```

Supplementary Material S2

R code for Cohens kappa:

```
library(tidyverse)
library(readxl)


natinfewe <- read_excel("M:/Lab results/R stats spikedFEC/Revision
documents/nat_infected.xlsx",      sheet = "kap-ewe")
natinfcow <- read_excel ("M:/Lab results/R stats spikedFEC/Revision
documents/nat_infected.xlsx", sheet = "kap-cow")

# For kappa:
library("irr")
# Kappa ewes:
data(natinfewe)
kappa2(natinfewe[,2:3])

#Kappa cows
data(natinfcow)
kappa2(natinfcow[,2:3])
```

Supplementary Table S2: Raw data, number of eggs recovered from artificially spiked sheep and cattle faeces using Flukefinder® and the Becker sedimentation methods.

Species	Method	EPG	eggsinsample	eggsrecovered
cattle	Flukefinder	2	4	0
cattle	Flukefinder	2	4	3
cattle	Flukefinder	2	4	0
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	0
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	3
cattle	Flukefinder	2	4	0
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	4
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	1
cattle	Flukefinder	2	4	0
cattle	Flukefinder	2	4	0
cattle	Flukefinder	5	10	3
cattle	Flukefinder	5	10	2
cattle	Flukefinder	5	10	5
cattle	Flukefinder	5	10	5
cattle	Flukefinder	5	10	4
cattle	Flukefinder	5	10	4
cattle	Flukefinder	5	10	3
cattle	Flukefinder	5	10	3
cattle	Flukefinder	5	10	2
cattle	Flukefinder	5	10	4
cattle	Flukefinder	5	10	6
cattle	Flukefinder	5	10	6
cattle	Flukefinder	5	10	2
cattle	Flukefinder	5	10	3
cattle	Flukefinder	5	10	3
cattle	Flukefinder	5	10	5
cattle	Flukefinder	5	10	2
cattle	Flukefinder	5	10	4
cattle	Flukefinder	5	10	7
cattle	Flukefinder	5	10	2
cattle	Flukefinder	10	20	6
cattle	Flukefinder	10	20	14
cattle	Flukefinder	10	20	5
cattle	Flukefinder	10	20	16
cattle	Flukefinder	10	20	6

cattle	Flukefinder	10	20	13
cattle	Flukefinder	10	20	4
cattle	Flukefinder	10	20	4
cattle	Flukefinder	10	20	5
cattle	Flukefinder	10	20	5
cattle	Flukefinder	10	20	6
cattle	Flukefinder	10	20	11
cattle	Flukefinder	10	20	8
cattle	Flukefinder	10	20	12
cattle	Flukefinder	10	20	6
cattle	Flukefinder	10	20	8
cattle	Flukefinder	10	20	12
cattle	Flukefinder	10	20	13
cattle	Flukefinder	10	20	10
cattle	Flukefinder	10	20	9
cattle	Flukefinder	20	40	18
cattle	Flukefinder	20	40	19
cattle	Flukefinder	20	40	12
cattle	Flukefinder	20	40	20
cattle	Flukefinder	20	40	7
cattle	Flukefinder	20	40	14
cattle	Flukefinder	20	40	12
cattle	Flukefinder	20	40	14
cattle	Flukefinder	20	40	10
cattle	Flukefinder	20	40	12
cattle	Flukefinder	20	40	18
cattle	Flukefinder	20	40	12
cattle	Flukefinder	20	40	17
cattle	Flukefinder	20	40	18
cattle	Flukefinder	20	40	16
cattle	Flukefinder	20	40	17
cattle	Flukefinder	20	40	15
cattle	Flukefinder	20	40	14
cattle	Flukefinder	20	40	15
cattle	Flukefinder	20	40	12
cattle	Becker	5	25	0
cattle	Becker	5	25	2
cattle	Becker	5	25	2
cattle	Becker	5	25	2
cattle	Becker	5	25	0
cattle	Becker	5	25	0
cattle	Becker	5	25	1
cattle	Becker	5	25	0
cattle	Becker	5	25	1
cattle	Becker	5	25	1
cattle	Becker	5	25	0
cattle	Becker	5	25	0
cattle	Becker	5	25	1
cattle	Becker	5	25	2
cattle	Becker	5	25	0

cattle	Becker	5	25	0
cattle	Becker	5	25	2
cattle	Becker	5	25	0
cattle	Becker	5	25	0
cattle	Becker	5	25	3
cattle	Becker	10	50	2
cattle	Becker	10	50	2
cattle	Becker	10	50	5
cattle	Becker	10	50	3
cattle	Becker	10	50	2
cattle	Becker	10	50	4
cattle	Becker	10	50	4
cattle	Becker	10	50	1
cattle	Becker	10	50	2
cattle	Becker	10	50	4
cattle	Becker	10	50	2
cattle	Becker	10	50	2
cattle	Becker	10	50	1
cattle	Becker	10	50	3
cattle	Becker	10	50	5
cattle	Becker	10	50	1
cattle	Becker	10	50	2
cattle	Becker	10	50	2
cattle	Becker	10	50	4
cattle	Becker	10	50	3
cattle	Becker	20	100	2
cattle	Becker	20	100	3
cattle	Becker	20	100	2
cattle	Becker	20	100	7
cattle	Becker	20	100	2
cattle	Becker	20	100	3
cattle	Becker	20	100	4
cattle	Becker	20	100	4
cattle	Becker	20	100	4
cattle	Becker	20	100	4
cattle	Becker	20	100	1
cattle	Becker	20	100	9
cattle	Becker	20	100	6
cattle	Becker	20	100	1
cattle	Becker	20	100	2
cattle	Becker	20	100	2
cattle	Becker	20	100	7
cattle	Becker	20	100	6
cattle	Becker	20	100	5
cattle	Becker	20	100	1
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	1

sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	3
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	2
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	1
sheep	Flukefinder	2	4	0
sheep	Flukefinder	2	4	0
sheep	Flukefinder	5	10	4
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	5
sheep	Flukefinder	5	10	4
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	3
sheep	Flukefinder	5	10	4
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	4
sheep	Flukefinder	5	10	4
sheep	Flukefinder	5	10	3
sheep	Flukefinder	5	10	3
sheep	Flukefinder	5	10	3
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	2
sheep	Flukefinder	5	10	3
sheep	Flukefinder	5	10	3
sheep	Flukefinder	5	10	4
sheep	Flukefinder	10	20	10
sheep	Flukefinder	10	20	6
sheep	Flukefinder	10	20	6
sheep	Flukefinder	10	20	5
sheep	Flukefinder	10	20	5
sheep	Flukefinder	10	20	6
sheep	Flukefinder	10	20	9
sheep	Flukefinder	10	20	8
sheep	Flukefinder	10	20	5
sheep	Flukefinder	10	20	6
sheep	Flukefinder	10	20	6
sheep	Flukefinder	10	20	7
sheep	Flukefinder	10	20	6
sheep	Flukefinder	10	20	5
sheep	Flukefinder	10	20	6

sheep	Flukefinder	10	20	7
sheep	Flukefinder	10	20	8
sheep	Flukefinder	10	20	7
sheep	Flukefinder	10	20	7
sheep	Flukefinder	10	20	9
sheep	Flukefinder	20	40	17
sheep	Flukefinder	20	40	13
sheep	Flukefinder	20	40	15
sheep	Flukefinder	20	40	14
sheep	Flukefinder	20	40	18
sheep	Flukefinder	20	40	13
sheep	Flukefinder	20	40	17
sheep	Flukefinder	20	40	11
sheep	Flukefinder	20	40	10
sheep	Flukefinder	20	40	12
sheep	Flukefinder	20	40	13
sheep	Flukefinder	20	40	13
sheep	Flukefinder	20	40	13
sheep	Flukefinder	20	40	9
sheep	Flukefinder	20	40	12
sheep	Flukefinder	20	40	11
sheep	Flukefinder	20	40	11
sheep	Flukefinder	20	40	14
sheep	Flukefinder	20	40	14
sheep	Flukefinder	20	40	12
sheep	Becker	5	25	3
sheep	Becker	5	25	0
sheep	Becker	5	25	1
sheep	Becker	5	25	2
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	1
sheep	Becker	5	25	2
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	5	25	1
sheep	Becker	5	25	0
sheep	Becker	5	25	0
sheep	Becker	10	50	1
sheep	Becker	10	50	1
sheep	Becker	10	50	0
sheep	Becker	10	50	1
sheep	Becker	10	50	2

sheep	Becker	10	50	0
sheep	Becker	10	50	2
sheep	Becker	10	50	0
sheep	Becker	10	50	1
sheep	Becker	10	50	3
sheep	Becker	10	50	1
sheep	Becker	10	50	2
sheep	Becker	10	50	1
sheep	Becker	10	50	1
sheep	Becker	10	50	0
sheep	Becker	10	50	0
sheep	Becker	10	50	1
sheep	Becker	10	50	0
sheep	Becker	10	50	1
sheep	Becker	10	50	2
sheep	Becker	20	100	2
sheep	Becker	20	100	7
sheep	Becker	20	100	2
sheep	Becker	20	100	3
sheep	Becker	20	100	6
sheep	Becker	20	100	3
sheep	Becker	20	100	4
sheep	Becker	20	100	3
sheep	Becker	20	100	2
sheep	Becker	20	100	4
sheep	Becker	20	100	2
sheep	Becker	20	100	6
sheep	Becker	20	100	2
sheep	Becker	20	100	3
sheep	Becker	20	100	1
sheep	Becker	20	100	2
sheep	Becker	20	100	1
sheep	Becker	20	100	3
sheep	Becker	20	100	1
sheep	Becker	20	100	3

Supplementary Table S3: Samples from naturally infected ewes and cows. The samples were classified as fluke egg positive (1) or fluke egg negative (0).

ewe	ff	bec
1	0	0
2	0	0
3	1	1
4	0	0
5	1	1
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	1	0
15	0	0
16	0	0
17	1	0
18	1	0
19	1	0
20	0	0

cow	ff	bec
1	0	0
2	1	0
3	0	0
4	0	0
5	0	0
6	1	0
7	0	0
8	0	0
9	1	1
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	1	1
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0